Reproductive Biotechnology and Gene Mapping: Tools for Conserving Rare Breeds of Livestock

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Contents

Today's livestock diversity originated from the wild ancestor species and was subsequently shaped through the processes of mutation, genetic drift, and natural and human selection. Only a subset of the diversity present in the ancestral species survives in the domestic counterparts. A 2007 report released by UN Food and Agriculture Organization '*The State of the World's Animal Genetic Resources*', compiled from surveys conducted in 169 countries, found that nearly 70% of the world's remaining livestock breeds live in developing countries. The UN report was presented to more than 300 policy makers, scientists, breeders, and livestock keepers at the First International Technical Conference on Animal Genetic Resources, held in September 2007 in Interlaken, Switzerland. The conference aims were to adopt a global plan of action for conserving animal genetic resources as its main outcome. In this paper, the current and potential contributions of reproductive and molecular biotechnology are considered as tools of conserving rare breeds of livestock.

Introduction

Of the 50 000 known mammalian and avian species, a relatively small proportion has been domesticated. Approximately 40 livestock species, shaped by a long history of domestication and development, contribute to today's agriculture and food production. Livestock breed development has been and continues to be a dynamic process of genetic change driven by selection pressures, including environmental factors and human intervention through controlled breeding and husbandry, which has resulted in a great variety of genetically distinct breeds. The livestock breeds developed over thousands of years has, until recently, caused a net increase in genetic diversity over time. During the past 100 years, however, there has been a net loss of diversity because of an increased rate of extinction of livestock breeds and varieties [UN Food and Agriculture Organization (FAO) (2007)]. The number of breeds lost over the past 8 years is rapidly approaching the rate of extinction that occurred from 1900 to 1999 (Table 1). Losses have been accelerated by the rapid intensification of livestock production, a failure to evaluate local breeds, and inappropriate breed replacement or cross-breeding facilitated by the availability of high performing breeds (FAO 2007). As an example of inappropriate breed replacement, Uganda's indigenous, drought-hardy Ankole cattle could face extinction within 20 years because they are being rapidly supplanted by Holstein-Friesians, a breed which produces much more milk. During a recent drought, however, farmers who had Ankole cattle were able to walk them long distances to reach water sources while those who had traded the Ankole for imported breeds lost entire herds. The physiology and grazing behaviour of the imported breeds typically are not adapted to the natural pastures and climate of Africa, especially when drought strikes (Kay 1997). In another example, cross-breeding has almost decimated purebred populations of the East African Red Maasai sheep, which is renowned for its disease resistance to gastrointestinal parasites and high productivity under extremely challenging environments. In the mid-1970s, as a result of a subsidized dissemination program, many farmers in Kenya cross-bred their Red Maasai flocks with the less-hardy Dorpers sheep, which subsequently proved unsuitable in many production areas. In 1992, the International Livestock Research Institute (ILRI) undertook an extensive search in Kenya and northern parts of the United Republic of Tanzania, and was only able to locate a very small number of purebred animals, which later showed some levels of genetic contamination (Gibson and Candiff 2000).

The extinction of a breed or population means the loss of its unique adaptive attributes, which are under the control of many interacting genes and are the results of complex interactions between the genotype and the environment. Figure 1 illustrates the current status of livestock breeds (FAO 2007). The regions with the highest proportion of their breeds classified as at risk are Europe (28% of mammalian breeds; 49% of avian breeds) and North America (20% of mammalian breeds; 79% of avian breeds). Europe and North America are the regions that have the most highly specialized livestock industries, in which production is dominated by a small number of breeds. In recent years, many of the world's small farmers have abandoned their traditional animals in favour of higher yielding stock imported from Europe and the USA. For example, in 1994 local breeds comprised 72% of the sow population in northern Vietnam; within 8 years, however, this figure had dropped to just 26%. Of the country's 14 local pig breeds, five are now listed as vulnerable, two are considered critical state and three are facing extinction.

The 2007 report, '*The State of the World's Animal Genetic Resources*', compiled by the FAO, with contributions by the ILRI and other research groups, surveyed farm animals in 169 countries. Nearly 70% of the entire world's remaining unique livestock breeds are found in developing countries, which as described above, are at risk from the importation and farming of exogenous livestock breeds. Renowned organizations such as the ILRI and FAO have spearheaded the first important step for conservation by conducting livestock

Table 1. Livestock breed extinction rates over time

Year span	Number of breeds	Percent of breed
Before 1900	15	2
1900–1999	111	16
After 1999	62	9
Unspecified[a]	502	73
Total	690	100

[a]Unspecified = no year of extinction indicated. Adapted from FAO (2007).

breed surveys. These surveys are time-consuming and logistically complex; however, ILRI scientists have developed and integrated the Domestic Animal Genetic Resources Information System with the FAO's Domestic Animal Diversity Information System to streamline the process. These web-based information systems provide a means for curation and dissemination of valuable information that will support development of conservation priorities, as well as provide a database for use of reproductive and molecular biotechnologies to maintain valuable genetic resources.

Impact of Reproductive Biotechnology on Livestock Conservation

Modern reproductive biotechnologies, such as artificial insemination, embryo transfer, *in vitro* fertilization, gamete/embryo micromanipulation, semen sexing, genome resource banking and somatic cell nuclear transfer (cloning) have enormous potential for conserving rare breeds of livestock. The advent of artificial insemination in the 1940s with cattle and semen cryopreservation in the 1950s with poultry was instrumental in the successful transfer of genetic material between and among livestock populations and breeds. There are several anec-

dotal and published reports of applying these technologies to conserving rare livestock breeds. The Rare Breeds Program in Colonial Williamsburg, VA (USA) makes use of semen cryopreservation for livestock breeds dating from the 17th and 18th centuries, including Devon cattle, Leicester sheep, Ossabaw pigs and American Cream horses, although actual numbers of offspring from artificial insemination with frozen/thawed semen have not been documented to date. The Hamilton Rare Breeds Foundation in Hartland, VT (USA) has pioneered in the use of frozen semen in the Poitou Donkey, an ancient breed dating back over 2000 years, and is the first group to have produced foals from the rarest breed of donkey in existence today.

Embryo transfer, pioneered in agricultural species in the 1930s, also has been reported in use with heritage livestock breeds. In collaboration with the Swiss Village Farm (SVF) Foundation in Newport, RI (USA), frozen/thawed embryos from the Tennessee Myotonic or fainting goat breed were surgically transferred into two surrogate Nubian does (a common domestic breed) and resulted in the birth of one healthy buck (Matsas et al. 2005). In 2006, the SVF Foundation reported a second birth from interspecies embryo transfer: an endangered Gulf Coast lamb born to a Santa Cruz ewe. The Gulf Coast sheep provides a good example for the importance of preserving the unique genetic attributes of heritage livestock breeds, as the Gulf Coast is sheep is extremely resistant to parasites and nearly impervious to the foot rot that plagues many other ovine breeds.

One of the most promising areas of reproductive biotechnology is the creation of genetic resource banks as a conservation tool for rare livestock breeds. The concept of banking gametes, embryos and DNA material for conservation purposes is not new, as the idea has

Risk status of the world's mammalian breeds in January 2006: absolute (table) and percentage (chart) figures by region

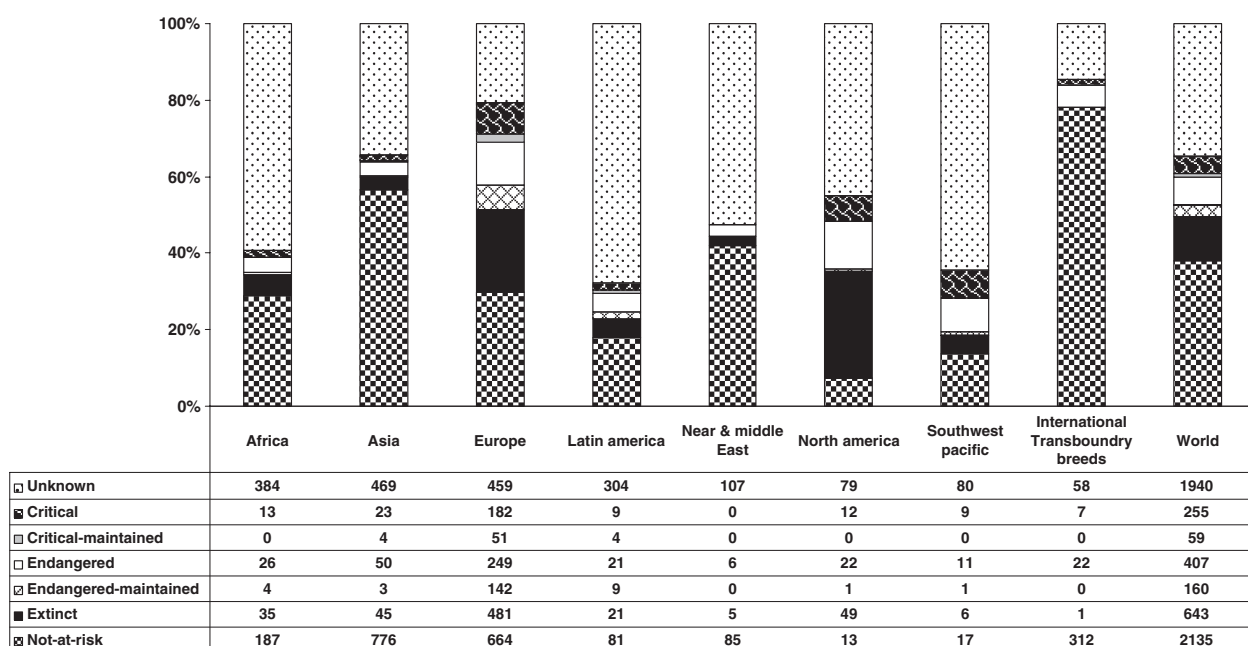|	Africa	Asia	Europe	Latin america	Near & middle East	North america	Southwest pacific	International Transboundry breeds	World
Unknown	384	469	459	304	107	79	80	58	1940
Critical	13	23	182	9	0	12	9	7	255
Critical-maintained	0	4	51	4	0	0	0	0	59
Endangered	26	50	249	21	6	22	11	22	407
Endangered-maintained	4	3	142	9	0	1	1	0	160
Extinct	35	45	481	21	5	49	6	1	643
Not-at-risk	187	776	664	81	85	13	17	312	2135

Fig. 1. Proportion of the world's breeds by risk status category. Adapted from FAO (2007)

Table 2. Rare and historic breed livestock genetic resource banks

Name	Country
Avian Resource Center	Canada/British Columbia
Center for Genetic Resources	The Netherlands
National Bureau of Animal Genetic Resources	India
INRA's National Cryobank	France
International Livestock Research Institute	Kenya
Livestock Research Institute	Taiwan
Nordic Genebank for Farm Animals	Norway
Rare Breeds Gene Bank	New Zealand
Swiss Village Farm Foundation	USA
USDA's National Animal Germplasm Program	USA

been widely discussed for use in preserving endangered wildlife populations (Wildt 1992, 2000; Long et al. 1996; Wildt et al. 1997; Holt and Pickard 1999; Andrabi and Maxwell 2007). Table 2 lists the pre-dominant germplasm/genetic repositories for rare livestock breeds around the world. One notable success story that illustrates the potential benefits of genetic resource banks involves the Dutch Friesian cattle breed. In 1879, the cattle population in the province of Friesland consisted mainly of Red Pied cattle registered as a red and white phenotype in the Friesian Cattle herd book. Black and white cattle progressively became more popular than the original red and white; by 1970, there were only 50 farmers registered as owning a total of 2500 Red and White Friesian cattle. The sustained import of Holstein-Friesians from the United States and Canada further eroded the population, to where only 21 Red and White cattle (4 males and 17 females) remained in 1993. A group of owners started the Foundation for Native Red and White Friesian Cattle and, in collaboration with the Nordic Genebank for Animals, developed a breeding program. Frozen/thawed semen that was preserved the 1970s and 1980s and subsequently stored in the Genebank was used to breed females under a contract system. Resulting male progeny were raised by breeders, and semen from these males was collected, frozen and later used under new contracts. The breed increased in number, reaching 256 registered living females and 12 living males in 2004. Currently, a total of 11 780 semen doses from 43 bulls are stored in the Genebank and kept available for artificial insemination (FAO 2007). Another example of successful usage of semen from storage repositories involves the endangered Gauloise dorée chicken, the oldest patrimonial poultry breed in France. Using frozen/thawed semen and an intensive breeding program, current stocks have proven the restoration of more than 96% of the initial genome (Blesbois et al. 2007). This point is particularly important for avian breeds, as neither the female gamete nor embryo has not been successfully cryopreserved and, unlike mammals where the male gamete determines gender, birds have a ZZ male/ZW female sex-determining system.

More recent reproductive biotechnologies such as somatic cell nuclear cloning also have enormous potential for conserving rare breeds of livestock. Another rare breed success story involves the cloning of the Enderby Island Cow, the last survivor of the world's rarest cattle breed. In 1992, members of New

Zealand's Rare Breeds Conservation Society found fresh hoof-prints of two cattle on Enderby Island. Five months later, the world's only surviving Enderby Island cow and her heifer calf were captured. Unfortunately, the calf subsequently died of unknown causes, leaving the cow as the only survivor of her breed in the world. A total of 35 embryo transfers were conducted, and resulted in the birth of a single male calf. In a last effort to save the breed, somatic cell nuclear transfer was used to produce heifer clones from the cow (Wells et al. 1998). To date, two of the three surviving clones have since given natural birth to two heifer calves.

Somatic cell cloning also was used to produce live offspring from the rare European mouflon sheep, a breed found on Sardinia, Corsica and Cyprus where there is thought to be fewer than 1000 mature individuals in the wild. Loi et al. (2001) injected enucleated sheep oocytes from a closely-related domestic breed with somatic granulosa cells recovered from the ovaries of two adult female mouflons found dead in the pasture. Blastocyst-stage cloned embryos were transferred into sheep foster mothers and two pregnancies were established, one of which produced an apparently normal mouflon lamb. What is remarkable about this example is that although the nuclear donor cells were recovered from dead animals and considered non-viable, these post-mortem cells were able to generate normal embryos and offspring. This example supports the use of cloning for the expansion of critically endangered populations, both within a concerted conservation program and in extreme situations involving sudden death (Loi et al. 2001). Despite these reports of the positive impact of reproductive biotechnology on the conservation of rare livestock breeds, there are too few examples of artificial insemination, germplasm cryopreservation, or embryo transfer being used in conjunction with rare livestock.

Application of Molecular Biotechnology Tools for Livestock Conservation

At the molecular level, the genetic diversity present within a livestock species is a reflection of differences in DNA sequences, or allelic diversity, across the functional DNA regions, or genes affecting animal development and performance. The complete and partial sequencing of major livestock genomes (chicken, 2004; bovine, 2005; rabbit, 2006; pig, 2007) provides a wealth of information useful for many aspects of livestock breed conservation from identifying ancestral breeds to understanding disease resistance.

Gene mapping has been used to as a tool to understand livestock origin and diversity in several livestock species. For example, 5 distinct maternal mitochondrial major lineages have been identified in domestic goats (Luikart et al. 2001; Sultana et al. 2003; Joshi et al. 2004); while the Asian mouflon is purported to be the only progenitor of domestic sheep (Hiendleder et al. 1998). The ancestor of the domestic pig is the wild boar (Sus scrofa), with at least 16 distinct subspecies of wild boar have been described in Eurasia and North Africa. A recent survey of mitochondrial DNA diversity among Eurasian domestic pigs and wild boar revealed a complex picture of pig domestication, with at least five

or six distinct centres across the geographical range of the wild species (Larson et al. 2005).

Domestication of cattle has been particularly well documented through gene mapping, with clear evidence of three distinct initial domestication events for three distinct aurochs (*Bos primigenius*) subspecies. *Bos primigenius primigenius* and *B. p. opisthonomous*, are the ancestors of the humpless *B. taurus* cattle of the Near East and Africa, respectively, with domestication occurring approximately 9000 years ago (Wendorf and Schild 1994). Humped Zebu cattle (*B. indicus*) are believed to have been domesticated at a later date, approximately 7000–8000 years ago (Loftus et al. 1994; Bradley et al. 1996; Bradley and Magee 2006). Finally, the domestic chicken (*Gallus domesticus*) is descended from the wild red jungle fowl (*G. gallus*). While previous molecular studies suggested a single domestic origin in Southeast Asia (Fumihito et al. 1994, 1996), at least six distinct maternal genetic lineages have now been identified (Liu et al. 2006).

In genetic diversity studies, the most frequently used markers are microsatellites and these are the most popular markers in livestock genetic characterization studies (Sunnucks 2001). Their high mutation rate and co-dominant nature permit the estimation of within- and between-breed genetic diversity, and genetic admixture among closely related breeds. There are a few examples of large-scale analyses of the genetic diversity of livestock species. For example, chicken and pig diversity throughout Europe have been reported (Hillel et al. 2003; SanCristobal et al. 2006). Sheep diversity was assessed at a large regional scale in northern European countries (Tapio et al. 2005); while Cañon et al. (2006) studied goat diversity in Europe and the Middle East. Probably the most comprehensive study of this type in livestock is a continent-wide study of African cattle (Hanotte et al. 2002), which revealed the genetic signatures of the origins, secondary movements and differentiation of African cattle. For most livestock breeds, however, a comprehensive review is still lacking.

Single nucleotide polymorphisms (SNPs) are used as an alternative to microsatellites in genetic diversity studies (Marsjan and Oldenbroek 2007). Single nucleotide polymorphisms are variations at single nucleotides which do not change the overall length of the DNA sequence in the region and occur throughout the genome. With this perspective, large-scale projects are ongoing in several livestock species to identify millions (Wong et al. 2004) and validate several thousands of SNPs, and identify haplotype blocks in the genome.

Mitochondrial DNA (mtDNA) polymorphisms have been extensively used in phylogenetic and genetic diversity analyses. The haploid mtDNA, carried by the mitochondria in the cell cytoplasm, has a maternal mode of inheritance (individuals inherit the mtDNA from their dams and not from their sires) and a high mutation rate; it does not recombine. These characteristics enable biologists to reconstruct evolutionary relationships between and within species by assessing the patterns of mutations in mtDNA. MtDNA markers may also provide a rapid way of detecting hybridization between livestock species or subspecies (Nijman et al. 2003).

An alternative approach to the identification of genome regions carrying relevant genes has recently been proposed. It consists of the detection of 'selection signatures' via a 'population genomics' approach (Black et al. 2001; Luikart et al. 2003). Population genomics utilizes phenotypic data at the breed level (or subpopulations within a breed), rather than at the individual level. The population genomics approach also can identify genes subjected to strong selection pressure and eventually fixed within breeds and, in particular, genes involved in adaptation to extreme environments or disease resistance. Population genomics relies on the principle that loci across the genome are influenced by genome-wide evolutionary forces (e.g. genetic drift, gene flow), whereas locus-specific forces, such as selection, imprint a particular pattern of variability on linked loci only (Luikart et al. 2003). By comparing the genetic diversity of many loci across the genome, it is then possible to reveal loci displaying an atypical variation pattern, which are likely to be linked to those genomic regions affected by selection (Black et al. 2001). Therefore, in contrast to candidate-gene-based methods, strategies making use of population genomics do not focus on a few loci only, but rather depict the effect of selection over the whole genome (Storz 2005).

Another new frontier emerging from the concept of population genomics is landscape genomics. Livestock by definition are adapted to the landscape (e.g. temperature, altitude, rainfall, disease challenge, nutritional challenge and human selection). The aim of landscape genomics is to learn from the co-evolution of livestock and production systems and use the knowledge gained to better match different breeds with production circumstances. A novel approach for evaluating population genomics is based on a spatial analysis method designed to detect signatures of natural selection within the genome of domestic and wild animals (Joost et al. 2007). Spatial analysis method goes a step further compared to classical approaches, as it is designed to identify environmental parameters associated with selected markers (FAO 2007). By overlaying population genomic analyses (e.g. 'signatures of selection') with other sets of information such as agro-ecological maps or other environmental/production information, it can be determined what genetic materials are candidates for use in which parts of the globe. The concept of landscape genomics is promising, as this combines georeferencing of breed distributions, spatial/global genetic diversity, climatic, ecological, epidemiological and production system information which will facilitate and direct priority decisions for breed conservation.

Future Challenges and Opportunities

Lack of information on the world's livestock resources, such as what livestock breeds/populations exist, their geographical location and their genetic characteristics, is a major impediment to their sustainable use. The current documented numbers of breeds is likely an underestimation, as a large proportion of indigenous livestock populations are in the developing world and have yet to be described at phenotypic and genotypic levels (Hanotte and Jianlin 2005). Additionally, the genetic

characterization of all remaining wild ancestral populations and closely related species is critical as these are the only remaining sources of putative alleles of economic values that might have been lost during domestication events. Moreover, the development and use of reproductive biotechnology, particularly genetic resource banks, is critical for the preservation and management of the remaining agricultural resources. There is a large gap between developed and developing countries in the ability to use reproductive and molecular biotechnology for setting and maintaining conservation priorities. The recent International Technical Conference on Animal Genetic Resources was a timely event that presented many areas for global concern and provided leadership for setting conservation priorities. It is particularly important to conserve the current livestock genetic resources because the ancestors of most of our existing livestock species no longer exist. Genetically diverse livestock populations provide a greater range of options for meeting future challenges, whether associated with environmental change, emerging disease threats, new knowledge of human nutritional requirements, fluctuating market conditions or changing societal needs.

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